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Blood and Merogonic Stages of *Haemogregarina* Species Naturally Infecting The Nile Monitor *Varanus niloticus*, Egypt

Abdel-Nasser Ahmed Hussein
Zoology Department, Faculty of Science, South Valley University, Qena, Egypt
E-mail: Hussein.ana@sci.svu.edu.eg

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ABSTRACT

Haemogregarines are haemoparasites (Apicomplexa: Adeleiorina) of all class of vertebrates nearly, worldwide. The present study was aimed to establish and describe the developmental stages of a *Haemogregarina* species infecting Nile monitor (*Varanus niloticus*). The captured Nile monitors were three animals with 80-100 cm in length, they captured from Qena province, Southern Egypt, and thin blood films were prepared and microscopically examined. Also, small pieces of the liver and the lungs of the infected animals were separated and prepared for sectioning, which stained and examined. The recovered parasites have rod-like bodies, infecting the erythrocytes only, with a single stage, in a parasitophorous vacuole and have no karyolitic effect. The parasitaemia level was found up to 50\10,000 counted erythrocytes. Only gamonts have been recognized in blood smears, and they were found in two forms; the first form is short, thick (called short gamont) and measured 11.02 × 2.65 µm. The second form was found long and thin (called long gamont), with a subterminal nucleus, measured 14.18 × 2.45 µm. Both forms cause either slightly or strong displacement of the host cell nucleus. The infected erythrocytes had either slightly or markedly hypertrophied in presence of short and long gamonts. The infected erythrocyte measured 18.05 × 8.87 µm, but the uninfected erythrocyte was 16.52 × 8.77 µm in length and width, respectively. Schizogony phase was observed in the lungs only of the infected Nile monitor, where the meronts were formed within parasitophorous vacuoles. The parasite nucleus divided many times, and the produced nuclei are peripherally arranged in the meront, forming merozoites. After separation of the merozoites, residual bodies formed. The merogonic stages were differentiated into two forms, micromeront that measured 15.57×14.29 µm, and produces a few numbers of macromerozoites, meanwhile the macromeront measured 19×15.9 µm and produces up to six micromerozoites.

INTRODUCTION

Importance of studying reptilian parasitic infections comes from their effections on evolution and ecology of the hosts (Smallridge and Paperna, 2000; Eisen, 2001). Nile monitor, *Varanus niloticus*, is the most large lizard species of class Reptilia. Generally, they have been observed to be infected with many internal such as protozoans and helminths (Wolf *et al.*, 2014), and external parasites (ticks and mites) (Pietzsch *et al.*, 2006).These parasites are of economic and social values (Odeniran *et al.*, 2016).

Reptilian parasitic infections remain less-known, despite this being important because parasitism causes deleterious effects on several aspects of the ecology and evolution of parasite hosts (Smallridge and Paperna, 2000; Eisen, 2001). Haemogregarines are apicomplexan blood parasites, cosmopolitan, intraerythrocytic with an obligatory heteroxenous life cycle (Smith 1996). The merogoric stages of these parasites are occurring in the cells of internal organs, and gamonts in erythrocytes of reptiles and other vertebrates. Sporogony phase takes place in the invertebrate hosts. Currently, haemogregarines are divided into four families (Barta *et al*., 2012), which are Dactylosomatidae Jakowska and Nigrelli, 1955, Haemogregarinidae Léger, 1911, Hepatozoidae Miller, 1908, and Karyolysidae Labbé, 1894.

Haemogregarines infecting reptiles are known through different taxonomic studies, but most of studies of haemogregarine parasitic infections in reptiles had been concerned with geckoes in Egypt (Bashtar *et al* ., 1984 a, b, &1987; Abdel -Ghaffar, 1985; Abdel - Ghaffar *et al*., 1994; Saoud *et al.,* 1995; Mohammed and Ramadan, 1996; Ahmed, 1998; El–Toukhy *et al.,* 2013; Rabie and Hussein, 2014; Cook *et al.,* 2016; Abou-Shafeey *et al.,* 2019). The Nile monitor (*Varanus niloticus*) has less attention in such studies in Egypt.

Wolbach (1914) in Lamin Kota in the Gambia has documented the first report of *Haemogregarina* parasites from Nile monitors. Later, came Ramadan *et al*. (1996) with a study about the protozoan blood parasites in *V. griseus* in Egypt, and recently, Al-Hoot and Abd-Al-Aal, 1999 described *Haemogregarina* species infecting the Nile monitor (*V. niloticus niloticus*) in Toshka, Upper Egypt.

The parasitaemia level of infections with haemogregarines in reptiles have been documented in many articles (Bashtar *et al.,* 1987; Hussein 2006; Al-Farraj 2008; Martínez-de la Puente and Merino, 2008; Galal 2010; Abdel-Baki and Al-Quraishy 2012)

Regarding to the affection of erythrocytes with infections, Wolbach (1914) observed very few hemogregarines in red corpuscle only, and recognised the displacement of the host cell nucleus. The gamonts of *H. roshdyi* affect the infected cells, where they were stretched and the nuclei displaced (Ramadan *et al.,* 1996). The erythrocytes of the Nile monitors infected with *Haemogregarina* species were hypertrophied and their nuclei were often pushed to the opposite side of the parasite (Al-Hoot and Abd-Al-Aal, 1999).

The merogonic stages of haemogregarines infecting Nile monitors have been observed in different internal organs; where various stages were found in the heart, lungs, liver, spleen, kidneys, and in the walls of the stomach and intestines (Wolbach 1914). But these stages have observed in the lungs and liver of *V. griseus* infected with *H. roshdyi* (Ramadan *et al.,* 1996). The schizogonic stages of these parasites were found in the endothelial cells of lungs capillaries (Al-Hoot and Abd-Al-Aal, 1999).

Due to the importance of reptiles and thier parasites, and lack of such knowledge about the parasitic profile of monitors in Egypt, this study investigated haemogregarines infecting the Nile monitors, *V. niloticus*.

MATERIALS AND METHODS

Study Area:

Qena Governorate is one of the Egyptian governorates in the southern part of the country; it covers a stretch of the Nile valley.

Animals:

Nile monitors had been captured and kept separately in cages with food and water at room temperature (28–35°C). The captured animals were three (one male and two females) and the length ranged from 80 to 100 cm.

Techniques:

Giemsa-stained blood films have made and examined using a light microscope. From lungs and liver of the

infected animal, small pieces (2 mm) have been cut, washed in 0.9% physiological saline, fixed in 3% glutaraldehyde buffered in 0.1 M sodium cacodylate (p^H 7.3), then washed in the same buffer, postfixed in 2% osmium tetroxide, washed in the buffer and dehydrated in ethanol. These specimens were embedded in araldite. Semithin sections were made on a Reichert microtome (thickness 0.5–0.7 µm), stained using methylene blue and examined by a Leica research photomicroscope. Ultrathin sections (40–50 nm) were prepared, stained and contrasted using lead citrate and uranyl acetate (Reynolds, 1963).The sections then examined with a JEOL transmission electron microscope (JEM-1010). The blood and tissue stages in the semithin section were observed and photographed using a Leica DM1000 microscope with a Leica EC3 camera. Measurements were made with an eyepiece micrometer. These measurements are given in micrometers (mean ± standard deviation followed by the range in parentheses). The present data have been compared with those previously described.

RESULTS

1- Blood stages and parasitaemia (Table 1; Figs. 1-3):

Examination blood smears of infected *V. niloticus*, different parasite's forms have observed. These stages were found infecting the erythrocytes only, where none of the leucocytes are infected. The parasites are usually intracellular and very few stages were observed extracellular (Fig. 1). The parasitophorous vacuole surrounding blood stages could recognize in long gamont using a light microscope (Fig. 3). Single parasite stage only was observed in the infected erythrocyte (Figs. 2&3). The parasitaemia level (number of parasite infections/10.000 erythrocytes counted) is up to 50 per 10,000 counted erythrocytes.

Only gamonts have been recognized in the blood smears. The gamont's body was rod-like in shape, and either one or two ends were slightly bent inward. These gamonts have differentiated into two forms; the first is short and thick form measuring 11.02 ± 0.94 × 2.65 ± 0.3 µm (range: 10.2-12.24 × 2.04-3.06 µm). The short gamont had round ends generally, the nucleus occupied less than half of the parasite, and parasite causes displacement of the host cell nucleus (Fig. 2). The second form is long and thin gamont, possessed round and curved ends, and the nucleus occupied about 1/4 of the body size, the nucleus of the parasite was central or subterminal in position. The long gamont measured 14.18 ± 0.75 × 2.45 ± 0.53 µm (range: 13.26-15.3 × 2.04-3.06 µm) (Fig. 3)**,** which causes a strong effect on the host cell. The parasite has no karyolitic effect.

Generally, the infected erythrocytes clearly affected by the presence of parasites, where the host cell showed hypertrophy and the nucleus had been pushed to the opposite side of the gamont. In case of long gamonts the infected cells had markedly hypertrophied. The infected cell measured 18.05 ± 0.97 × 8.87 ± 0.49 µm (range: 16.32-19.38 × 8.16-9.18 µm) and the uninfected one measured 16.52 ± 0.80 × 8.77 ± 0.53 µm (range: 15.3-17.34 × 8.16-9.18 µm) in length and width, respectively (Figs. 2&3).

2-Merogony and merozoites (**Table** 1; **Figs. 4-6):**

The parasites have lifted infected erythrocytes and attacked parenchyma cells in the liver, where parasites go into merogony or schizogony. Schizogony was observed in different developmental phases - in few numbers - in the endothelial cells of the blood capillaries in the lungs (Figs. 4-6). But there are no schizogonic stages observed in the liver of the infected Nile monitors. The parasitophorous vacuole appeared enclosing the parasite and grows in size during the growth of the meront. The formation of merozoites began with the division of the parasite nucleus into nuclei. Few daughter nuclei were seen peripherally arranged in the meront (Fig. 4). The cytoplasm remained, after the separation of the merozoites, appeared as a residual body. The nuclei of the multinucleated meront undergo elongation and finally formed merozoites (Figs. 5&6).

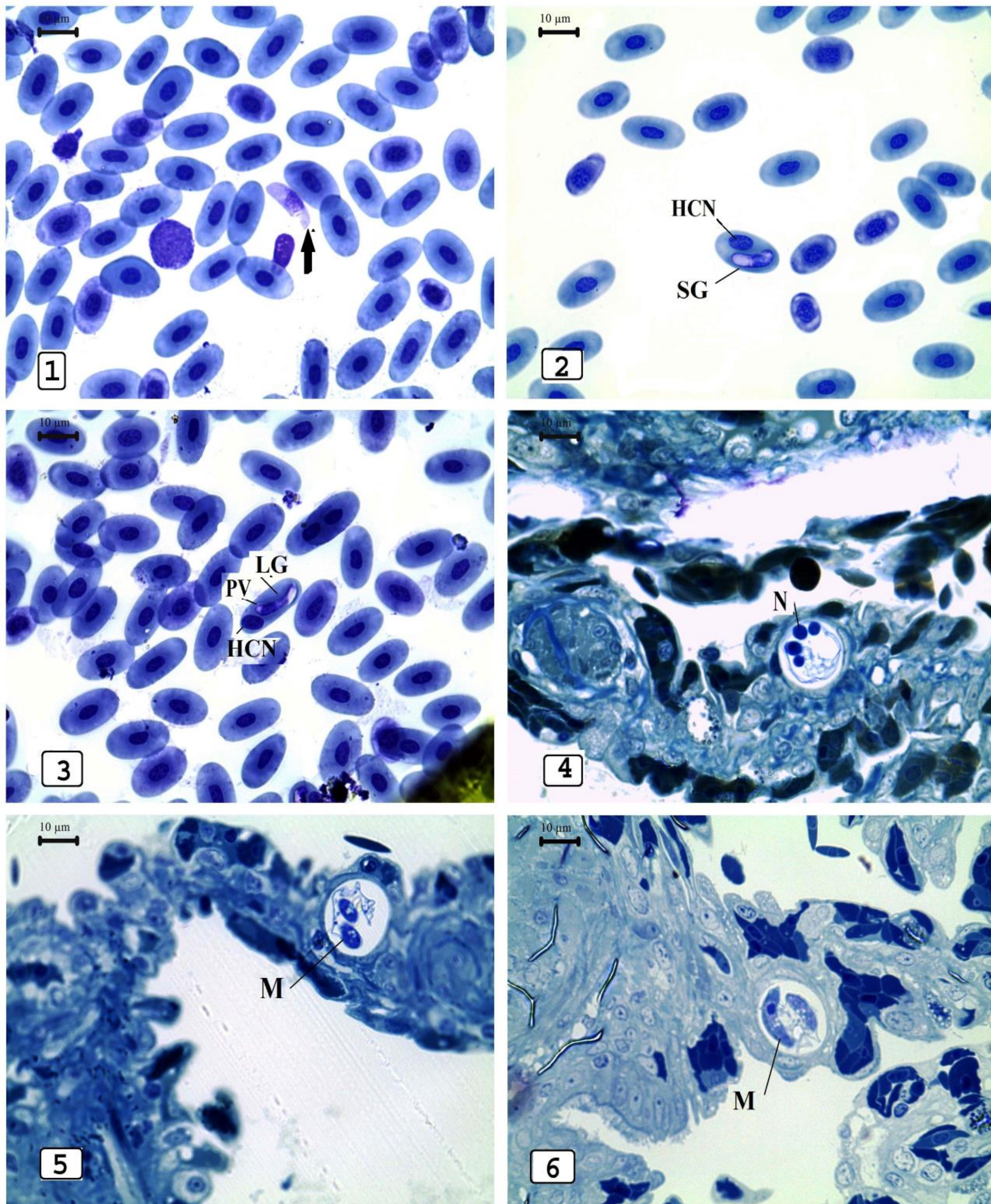
There are different merogonic stages in the lungs. According to the measurements, the meronts are present in two forms, small form named micromeront, measured 15.57±0.98×14.29 ± 0.49 µm (range: 15-17 × 14-15 µm) and produces a few numbers (2-3) of large merozoites (macromerozoites) (Figs. 5&6). Large form called macromeront that measured 19±1.05×15.9 ± 0.88 µm (range: 18-20 × 15-17 µm) and produces more (4-6) small size merozoites (micromerozoites) (Figs. 4).

Table 1: Measurements of the present haemogregarine infecting Nile monitores (*Varanus Niloticus*)

Normal B C		Infected B C		Short gamont		Long gamont		Micromeront		Macromeront	
L	W	L	W	L	W	L	W	L	W	L	W
15.3	9.18	16.32	9.18	12.24	3.06	14.28	3.06	15	15	20	17
16.32	9.18	17.34	9.18	12.24	3.06	13.26	2.04	17	14	18	15
17.34	8.16	17.34	9.18	10.2	3.06	14.28	2.04	15	14	18	15
16.32	9.18	18.36	8.16	10.2	2.04	14.28	3.06	15	14	20	15
16.32	8.16	18.36	8.16	12.24	3.06	15.3	3.06	17	15	18	16
17.34	9.18	17.34	9.18	10.2	3.06	14.28	2.04	15	14	20	17
16.32	8.16	19.38	9.18	10.2	3.06	15.3	3.06	15	14	20	15
17.34	9.18	18.36	9.18	11.22	2.04	13.26	2.04	-	-	18	16
15.3	8.16	18.36	9.18	10.2	2.04	13.26	2.04	-	-	20	16
17.34	9.18	19.38	8.16	11.22	2.04	14.28	2.04	-	-	18	17
Maen= 16.52	**8.77**	**18.05**	**8.87**	**11.02**	**2.65**	**14.18**	**2.45**	**15.57**	**14.29**	**19.00**	**15.90**
SD= 0.80	**0.53**	**0.97**	**0.49**	**0.94**	**0.53**	**0.75**	**0.53**	**0.98**	**0.49**	**1.05**	**0.88**

Table 2: Comparative descriptive data of the present species, with morphologically similar Haemogregarines species from monitors

Parasite\Reference	Host	Blood stages		Tissue stages	
		Stage	Measurement (µm)	Site	Measurement (µm)
Haemogregarine\ Wolbach 1914	*V. niloticus*	Large bodies	10.3× 2.5	Heart, Lung, Liver, Spleen, Kidneys, Stomach, Intestine	Schizont with single nucleus-- 7× 4 (micromeront)
		Small bodies	6 × 3		Schizont with many nuclei-- 15×5 (macromeront)
***Haemogregarina roshdyi* n.sp.\ Ramadan *et al.,* 1996**	*V. griseus*	Young trophozoite	8-13 × 2-3.5	Lungs Liver	Macromeront Rounded-25 in diameter Oval- 20-28.4×12-19
		Growing trophozoite.	10-18.5 × 3.5-5.6		Micromeront Rounded- 26 in diameter Oval– 27-36× 14.4-15
***H. varani*\ Laveran 1905 and Laveran and Pettit 1909(cited from Ramadan *et al.,* 1996)**	*V. niloticus*	Small	6-8 × 3	-	Micromeront 15-16 contains 4 macromerozoites
		Medium	8-11 × 3		
		Large	11-15 ×3		Macromeront 18-21 contains 32 micromerozoites
***Haemogregarina* sp.\ Al-Hoot & Abd-Al-Aal 1999**	*V. niloticus*	First	6.75 × 3.02	Lung	Smallest meront 7.45 × 6.22
		Second	9.07 × 3.72		Micromeront- 17.4 ×15.2
		Third	12.05 × 2.70		Macromeront- 32.9 × 22.13
***Haemogregarina* sp.\ Present study**	*V. niloticus*	Short gamont	11.02 × 2.65	Lung	Micromeront 15.57 ×14.29 produces 2-3 macromerozoites
		Long gamont	14.18 × 2.45		Macromeront 19 ×15.9 produces 4-6 micromerozoites



Figs. 1-3: Light micrographs of Giemsa stained blood smears of the Nile monitors *Varanus niloticus* naturally infected by *Haemogregarina* species showing: An extracellular gamont (arrow) (Fig. 1); Short gamont (SG), and slightly displaced host cell nucleus (HCN) (Fig. 2); Showing long gamont (LG) surrounded by parasitophorus vacuole (PV), and displaced host cell nucleus (HCN) (Fig. 3);

Figs. 4-6: Light micrographs of semi-thin sections in lungs of the Nile monitors *Varanus niloticus* naturally infected by *Haemogregarina* species showing different merogonic stages: A meront (macromeront) containing four daughter nuclei (N) (Fig. 4); A micromeront possesses two developing merozoites (M) (Fig. 5); A micromeront contains will developed merozoites (M) (Fig. 6).

DISCUSSION

Haemogregarines are apicomplexan paraites and heteroxenous life cycle, where gamonts infect red blood cells of reptiles and other vertebrates. Merogony (or schizogony) of haemogregarines occures in certain internal organs of vertebrate hosts, but sporogony takes place in invertebrate hosts. These blood parasites are included in the family Haemogregarinidae that contains four genera: *Haemogregarina*, *Hepatozoon* Miller, 1908, *Karyolysus* Labbe´, 1894 and *Cyrilia* Lainson, 1981 (Levine, 1988). The best differentiation method between haemogregarines, it has been suggested that these blood gregarines should be assigned to the genus *Haemogregarina* in its broad sense (Levine 1988; Abdel-Ghaffar *et al.,* 1994; Ramadan *et al.,* 1996, Majlathova´ *et al.,* 2010; Roca & Galdon, 2010). For differentiation of haemogregarines infecting the African monitor lizard, a molecular method reported by Cook *et al*. (2016) as the first morphological and molecular characterisation of a haemogregarine within the African Varanidae.

The previously published articles concerning haemogregarines infecting Nile monitors are very poor and too old enough to be disappeared. *H. varani* was originally described by Laveran (1905) in South Africa, and Senegal (Laveran and Pettit, 1909) (Table 2). So, according to Ramadan *et al.* (1996), three species of the genus *Haemogregarina* have been previously described from African monitores; *H. varani*, *H. borreli* and *H. toddy*. However, Ball (1967) suggested that *H. toddy* was probably a variant of *H. varani.* The gamonts of haemogregarines in different reptilian hosts had been differentiated into two forms (Abdel-Ghaffar *et al.,* 1994; Saoud *et al.,* 1995; Hussein, 2006; Rabie *et al.,* 2006 & 2014). Comparing the present data of *V. niloticus* with the previously described gamonts of *Haemogregarina* species, showed that of the present gamonts are different from those previously described in their measurements (Table 2). The in-hand study detects a low parasitaemia level (50\10,000) erythrocytes counted. Regarding parasitaemia level, in an infection of the skinks, *Mabuya quinquetaeniata*, Bashtar *et al.,* 1987 reported 40%-50% of the erythrocyte in infection with *Hepatozoon gracilis*, while Martínez-de La Puente and Merino (2008) stated a high parasitaemia level (1%-8%). In the same hosts infected with *Hepatozoon gracilis*, the highest parasitaemia level recorded was 11-20% per 1000 counted erythrocytes. (Galal, 2010). Al-Farraj (2008) reported 200-400 infected out of 1000 erythrocytes counted of a haemogregarine species infecting the viper *Cerastes cerastes gasperitti*. Regarding the affection of infected erythrocytes by parasites, the present study document they hypertrophied infected erythrocytes, and also the host cell nucleus displacement in presence of mature gamonts. Also, the present study didn't recognized karyolytic effect of gamonts on the host cell nucleus. This result agrees with those of Saoud *et al.* (1995); Hussein (2006); Al-Farraj (2008) and Abdel-Haleem *et al*. (2013).

In the early schizont, the nucleus begins to divides repeatedly, and the daughter nuclei migrate to the outer boundary. At the boundary membrane of the schizont, a thickening and protrusion appeared showing places of merozoites formation, where these structures are termed merozoite anlages. The same observations in merozoites formation were reported and described as ectomerogony by Bashtar *et al.* (1984a); Shazly (2000) and Hussein (2006). The present study ecognized the merogonic phase in the tissue of lung only, which agrees with Hussein, 2006; Abdel-Baki and Al-Quraishy 2012. Rabie *et al.* (2014) reported merogonic phase only in the liver of the Egyptian Bean Skinks. The previously published studies mentioned that the merogonic development of haemogregarines occurred in different organs of the infected vertebrate hosts (such as liver, spleen, lung, and kidney) (Ramadan *et al.,* 1996; Sakran *et al.,* 2006; Al-Ghamdy, 2011). The merogony of *Haemogregarina*

species infecting Nile monitors were found – commonly- in lungs and liver, but Wolbach (1914) mentioned merogony in Heart, Lung, Liver, Spleen, Kidneys, Stomach and Intestine. In the present study, the merogony was observed only in the endothelial cells of the blood capillaries in the lungs of infected *V. niloticus*. The present study recognized two forms of meronts, which considered a characteristic feature of haemogregarines and agreed with similar studies by Levine, 1988; Abdel-Ghaffar *et al.,* 1990; Saoud *et al.,* 1995; Smith and Desser, 1999; Mihalca *et al*., 2002). Generally, a comparison of previously described *Haemogregarina* species in African monitors with that in the present study showed the measurements and geographical distribution of the present gamonts and schizont of *Haemogregarina* species infecting *V niloticus* are different from those previously described (Table 2).

Conclusion

This study had been investigated and described gamogony and merogony of a haemogregarine infecting Nile monitor, in order to fill a gap in the biodiversity knowledge of haemogregarines infecting reptiles in Egypt and their host-parasite relationships. But when the way of parasite's transmission is still unknown, a description of gamogony and merogony is not enough to classify this parasite. Future experimental trials and molecular studies are urgent and recommended to fill this gap.

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